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Sumário

Avaliação de complexo homeopático e da zeólita clinoptilolita para a prevenção de diarréia em bezerros leiteiros do nascimento ao desmame

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Resumo – Avaliou-se a eficácia de um complexo homeopático e uma zeólita clinoptilolita na prevenção de diarreia em bezerros leiteiros. Trinta e sete bezerros foram distribuídos em três grupos: Controle negativo, Zeólita e Homeopatia. A composição da zeólita foi verificada por espectrometria de emissão óptica (ICP OES). Todos receberam tratamento alopático em caso de ocorrência de diarreia. As fezes foram avaliadas diariamente para observar a cor, o odor e a consistência. Os animais foram pesados e avaliados quanto à hidratação (teste de turgor cutâneo) semanalmente. O sangue foi coletado a cada duas semanas para aferição do volume globular e da proteína sérica total. Além disso, os custos dos tratamentos foram quantificados. Ao final do experimento seis animais de cada grupo foram eutanasiados para análise morfo-histológica por microscopia eletrônica de varredura de porções do intestino delgado. Considerando todos os animais e todos os episódios de diarreia, a duração média da diarreia foi de 2,42 dias, a idade média de ocorrência foi de 27,75 dias, o número médio de episódios por animal foi de 2,53 e o número médio de tratamentos com antibióticos por animal foi de 1,01, sem

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diferença entre os tratamentos. Durante os episódios de diarreia, não houve diferenças entre os grupos em relação à cor (86,60% verde), odor (79,40% anormal) e consistência (86,27% líquido) das fezes. O grupo de animais tratados com o complexo homeopático foi o único em que alguns animais não apresentaram episódio de diarreia durante o experimento (23,1%, $p \leq 0,05$). Embora o tratamento zeólita tenha preservado as vilosidades intestinais, não houve impacto na prevenção da diarreia. O complexo homeopático não teve impacto na duração da diarreia ou no ganho de peso dos bezerros. Embora sua adoção não altere os custos do sistema de produção, o produtor precisa levar em consideração se o manejo (preparação e administração diária) compensa o baixo efeito preventivo apresentado.

Termos para indexação: Bezerro em aleitamento; Complexo homeopático; Diarreia; Zeólita.

Evaluation of homeopathic complex and zeolite clinoptilolite for the prevention of diarrhea in dairy calves from birth to weaning

Abstract – The efficacy of a homeopathic complex and a zeolite clinoptilolite in the prevention of diarrhea in dairy calves was evaluated. Thirty-seven calves were distributed in three groups: Negative control, Zeolite and Homeopathy. The composition of the zeolite was ascertained by optical emission spectroscopy (ICP OES). All calves received allopathic treatment in case of diarrhea. The feces were evaluated daily regarding the color, the odor, and the consistency. The animals were weekly weighed and assessed regarding hydration (skin turgor test). Blood samples were collected every two weeks to measure globular volume and total serum protein. Furthermore, the costs of the treatments were quantified. At the end of the experiment, six animals from each group were euthanized for morpho-histological analysis by scanning electron microscopy of portions of the small intestine. Considering all animals and all episodes of diarrhea, the average duration of the diarrhea was 2.42 days, the average age of occurrence was 27.75 days, the average number of episodes per animal was 2.53, and the average number of antibiotic treatments per animal was 1.01, with no difference among the treatments. During the diarrhea episodes, there were no differences in the treatments regarding color (86.60% green), odor (79.40% abnormal), and consistency (86.27% liquid) of the feces. The group of animals treated with the homeopathic complex was the only one in which some animals did not present an episode of diarrhea during the experiment (23.1%, $p \leq 0.05$). The average duration of the diarrhea was 2.42 days, the average age of occurrence was 27.75 days, the average number of episodes per animal was 2.53, and the average number of antibiotic treatments per animal was 1.01, with no difference among the treatments. Although the zeolite treatment preserved the intestinal villi, there was no impact on diarrhea prevention. The homeopathic complex had no impact in the diarrhea duration or in the weight gain of calves. Although its adoption does not alter the costs of the production system, the farmer needs to consider whether the management (daily preparation and administration) compensates for low preventive effect presented.

Index terms: Suckling calves; Homeopathic complexes; Diarrhea; Zeolite.

Introduction

The colostrum-feeding phase is followed by the milk-feeding phase and this period is crucial to produce efficient dairy cows (Raeth-Knight et al., 2009; Moallem et al., 2010). Bovine neonatal diarrhea, however, is one of the main factors responsible for mortality and delayed development of calves (Hammon et al., 2002; Jasper; Weary, 2002). Diarrhea can be fatal due to dehydration and acidosis, and also can result in anorexia and ataxia. It usually results from the interaction of factors such as the immune system status, environmental conditions, nutrition and infection by various microorganisms (Berchtold, 2009), with the standouts being Escherichia coli, Salmonella spp., rotaviruses, coronaviruses and protozoa of the genera Eimeria spp. and Cryptosporidium spp. (Langoni et al., 2004; Cho; Yoon, 2014).

Diarrhea in Nelore calves is considered the main cause of economic losses in beef herds in various Brazilian States (Barbosa et al., 1998; Benesi, 1999; Mota et al., 2000), but is poorly documented in dairy herds in the country. More than 50% of the mortality that occurs before weaning is due to diarrhea, and the majority of cases happen in animals younger than one-month-old (Hur et al., 2013; Cho; Yoon, 2014). Antibiotics are typically used to treat diarrhea in livestock but are often used indiscriminately, causing resistance of pathogens and environmental contamination (Regitano; Leal, 2010; Economou; Gousia, 2015).

Therefore, new and natural alternatives to control pathogens in livestock are necessary (Prasai et al., 2017). Homeopathy is characterized as a safe and inexpensive practice aimed to treat the patient-disease binomial according to a comprehensive and integrative approach (Teixeira, 2006). Studies have evaluated different homeopathic complexes for prevention and treatment of diarrhea in calves in the suckling phase (Frerking; Romansky-Rieken, 1982; Kayne; Rafferty, 1994; Verdier; Ohagen; Alenius, 2003; Fortuoso et al., 2018), but the results are controversial. Zeolites are natural hydrated crystalline aluminosilicate minerals. They are structured in rigid three-dimensional crystalline networks formed by tetrahedrons of AlO_4 and SiO_4, linked together by oxygen atoms (Luna; Schuchardt, 2001). According to a review performed by Papaioannou et al. (2005), the inclusion of zeolites in the diet may reduce

the incidence, severity, and length of diarrhea in calves, but the precise mechanisms of this effects are yet not clear.

Therefore, the objective of this study was to evaluate the efficacy of using a homeopathic complex and zeolite clinoptilolite for the prevention of diarrhea in calves, as well as the effect on the animals' intestinal morpho-histology.

1 Material and Methods

1.1 Place and experimental groups

The experiment was conducted at the Milk Production System of the Embrapa Pecuária Sudeste research farm, located in the municipality of São Carlos, São Paulo state, Brazil. The calves were allocated in an area covering 0.2 ha, with an 'Argentine' calf pen structure, composed of five modules made of artificial shade screening (1.4 m wide x 48 m long x 1.6 m high), providing 80% shade. The modules were arranged in the North-South direction so that the animals had access to shade throughout the day. Each module was sized to accommodate 12 animals, which were tethered by collars with 1.2 - meter chains attached to wires anchored in the ground with 8 meters in length, arranged in the East-West direction. The wires anchored in the ground were spaced 4 meters apart, enough to prevent physical contact between the animals. The area was harvest with *Cynodon* grass, and there were water and food troughs arranged on the ground, one at each wire's anchor point.

A breeding season was programmed in order to have animals being born in a shorter time interval. Thus, Holstein and Jersey dairy calves, born in 2015 between February 10 and May 22, were allocated randomly in treatments as they were born. A total of 37 animals, 18 females and 19 males, were distributed in three groups: 1) Control (13 animals); 2) Homeopathy (13 animals); and 3) Zeolite (11 animals) and followed from 2 to 60 days of age. After birth, each calf was submitted separated from its mother for performance of the standard procedures (navel disinfection, weighing and identification tagging) and then was allocated in the cattle pen to receive colostrum (*ad libitum*). In the first week, the calves were bottle-fed with milk, after which they received milk from stainless steel buckets. The animals were provided with

clean water and ration, changed daily. From the second to the sixtieth day of life the animals received the following treatments:

- Negative control: 4 L of milk per day (2 L in the morning and 2 L in the afternoon);

- Zeolite: 4 L of milk per day + 10 g of zeolite diluted in the milk (2 L + 5 g in the morning and 2 L + 5 g in the afternoon);

- Homeopathy: 4 L of milk per day + 8 g of homeopathic complex diluted in the milk (2 L + 4 g in the morning and 2 L + 4 g in the afternoon).

1.2 Homeopathic treatment and establishment of the experimental protocol

The homeopathic complex was defined after the visit of a veterinarian to the farm, when a careful history-taking of the occurrence of diarrhea in the experimental area in the last years was carried out (number of days, period of occurrence, diarrhea characteristics, deaths/year, results of laboratory diagnostics, etc.). This process is called homeopathic repertorization, which guides the formulation of the specific complexes. Therefore, the preventive formulation was composed of *Arsenicum alb* 12 CH, *Nux vomica* 12CH, *Podophyllum* 12 CH, *Carbo Vegetabilis* 12CH, and *China* 12 CH. This formulation was given daily in the milk (8 g per day), and when an animal developed mild diarrhea, it was also administered orally by spray (3 times for 24h) to try to prevent it from getting worse. The homeopathic complex for inclusion in the milk was composed of 5 mL of the homogenized homeopathic formulation in 500 g of coarse sugar. After preparation, the product was stored in plastic pots.

The zeolite was a commercial product (CELPEC®) also administered in the milk. Since the mineral product is hard to dissolve in milk, to assure the proper consumption by each animal dilution tests were conducted with different concentrations, and the dosage of 0.25% zeolite obtained the maximum dilution without precipitation in the milk so that concentration was established in the following experiments.

In cases of diarrhea, the following protocol was implemented in all groups: supply of serum (1.5 g of salt + 10 g of sugar + 13 g of sodium bicarbonate + 1 L of water), given orally to all the animals twice a day, and allopathic

treatment if necessary. The cases were evaluated and treated individually by the veterinarian responsible for the herd. All the experimental procedures were approved by the Committee on Ethical Use of Animals (CEUA), under protocol 02/2014.

The feces of each animal were daily evaluated, to observe the color (yellow, white, gray, brown, green), odor (normal or abnormal) and consistency (liquid, pasty or hard). Each week the animals were weighed and the hydration was analyzed by the skin elasticity test (skin turgor test - STT) as: normal (0 seconds for return), slight (2 - 5 seconds for return), moderate (6 - 10 seconds), severe (more than 10 seconds for return). Each two weeks blood was collected to evaluate the globular volume and total serum protein. In cases of diarrhea, a notation was recorded of the duration and all the interventions carried out, to enable quantification of the animal treatment expense. The animals were observed during 60 days, after which they were weaned.

1.3 Morpho-histological evaluation of the calves' small intestine

At 60 days of age, six animals from each group were anesthetized and euthanized to collect samples of the small intestine. A segment with a length of 2 cm was removed from the three regions of the small intestine (duodenum, jejunum, and ileum). The tissues were fixed by immersion in a solution of 4% paraformaldehyde in sodium cacodylate buffer 0.1 M and sucrose 0.2 M, pH 7.2, for 2 hours, repeatedly washed with the buffer and prepared for electron microscope observation. Segments with a size of 4 x 4 mm were post-fixed in a 1% osmium tetroxide solution in sodium cacodylate buffer 0.1 M, pH 7.2, and dehydrated in rising concentrations of acetone. After drying to the critical point, the specimens were coated with gold and examined under a JEOL JSM-6510 scanning electron microscope, with magnification of 150, 300 and 500x for observation of villi and crypts.

1.4 Analysis of the zeolite composition

The zeolite was characterized by various analytical techniques. First, the particle size was assessed by a Sedigraf 5000 ET apparatus. Then the samples were microwave assisted decomposed in an Anton Parr Multiwave 3000 microwave oven (Vieira et al., 2005). The elemental content of the sample and the exchangeable cations were determined by inductively coupled plasma optical emission spectroscopy (ICP OES) with radial view (Varian Vista). The surface areas were measured by the BET (Brunauer-Emmett-Teller) method (Palik, 1977) using a Micrometrics ASAP 2000 V3.03 analyzer. The microscopic images were obtained with a Zeiss DSM 960 digital scanning electron microscope.

1.5 Statistical and economic analyses

The data on color, odor and consistency of the feces, as well as the hydration state, globular volume and total serum protein levels of the animals were organized in frequency tables, cross-referenced with the effect of the treatment group (Negative control, Homeopathy and Zeolite) and submitted to the t-test for comparison of the means ($p \leq 0.10$). The data on the fecal color, odor, and consistency during episodes of diarrhea were organized in the same form but were submitted to the Q-square test ($p \leq 0.05$). The data of length (number of days) and period of occurrence of diarrhea were submitted to analysis of variance, where treatment was the principal effect. The SNK test was applied for multiple comparisons of the means ($p \leq 0.05$).

In the second step of the analysis, the experimental unit was the calf, where the Fisher test was used to check whether the occurrence of diarrhea was associated with the treatment ($p \leq 0.05$). By combining the occurrence of diarrhea (Yes, No), four new groups were formed (Diarrhea Treatment: NegControl_Yes; Zeolite_Yes; Homeopathy_Yes; and Homeopathy_No). The data on the number of cases of diarrhea per animal, treatment with an antibiotic (number of treatments/animal), weight gain in the period, treatment and prevention costs and cost per live weight were submitted to analysis of variance considering the effect of the four new groups. For multiple comparisons of means, the SNK test was applied ($p \leq 0.05$). The SAS statistical software was used in all the calculations (SAS, 2012).

The experimental design was completely randomized, but the formation of the groups of calves by treatment was done according to the date of birth.

Results

Zeolites can be characterized by different techniques to determine their crystallinity, morphology, and chemical composition. Due to the complexity of the samples and the inherent limitation of each technique, it was necessary to conciliate them for better characterization of the zeolite used. The BET method revealed that the zeolite had a surface area of 22.62 m^2/g, the average pore diameter was 120.03 Å, and the pore volume was 0.07 cm^3/g. The X-ray diffractograms contained typical peaks given by the reflection angles (2θ). Therefore, concerning mineralogical composition, it was identified the presence of quartz, calcite, clinoptilolite, and kaolinite. The most representative peak for clinoptilolite was at an angle of 22.5°, with an intensity of 380 counts. For kaolinite, the most representative peak was at an angle of 10°, with an intensity of 480 counts. The chemical analysis of the zeolite showed the predominance of silicon (67.31%) and the presence of both Ca (6.55%) and Na (5.29%), the reason why heulandite/clinoptilolite was considered to be the predominant species of the zeolite. Heulandite has a composition of Ca_4 $(Al_8Si_{28}O_{72})$ $24H_2O$, while clinoptilolite's composition is $Na_6(Al_6Si_{30}O_{72})$ $24H_2O$.

The data after evaluation of the treatments for 60 days in relation to the negative control showed no significant difference between mean fecal color (green 77.57%, yellow 15.95%, brown 5.17%, white 1.17%, gray 0.13%), odor (normal 96.42%, abnormal 3.58%), consistency (pasty 91.97%, liquid 7.44%, hard 1.59%), general animal status (adequate 99.35%, apathetic 0.65%), animal hydration (normal in all cases, 100%), globular volume (31.97%) and total serum protein (6.67 g/dL). The information by experimental group is reported in Table 1. During the diarrhea episodes, there were also no differences between treatments regarding those characteristics, in which occurred predominance of the green color (average of 86.60% of the observations), abnormal odor (79.40%) and liquid consistency (86.27%) of feces.

The Homeopathic group was the only one in which some animals did not suffer from diarrhea ($p \leq 0.05$ by the Fisher test), with 23.1% of the animals' symptom-free (Table 2). In the other groups, all animals (100%) had at least one diarrhea episode during the experiment. Among the animals that had diarrhea, the average duration was 2.42 days, and the average age of occurrence was 27.75 days (Table 1). The average number of episodes per animal was 2.53, and the average number of antibiotic treatments per animal was 1.01, with no significant differences between the treatments (Table 2).

The average weight gain in the period was the same for all the animals, 25.06 ± 5,1 kg, including those that suffered from diarrhea in the period (Table 2). The cost of prevention and treatment of diarrhea and the cost per kilogram of weight gain in the period were higher in the animals of the Homeopathic group that had diarrhea (Homeopathy cost + antibiotic cost) than the animals of that group that were free of diarrhea. Prevention cost: Homeopathy_Yes, US$ 7.7 and Homeopathy_No, US$ 1.4; Cost per kilogram of weight gain: Homeopathy_Yes, US$ 0.3 and Homeopathy_No, US$ 0.1. Among the animals that had diarrhea, there were no differences between the treatments regarding cost per animal (US$ 6,01 on average) (Table 2).

Table 1. Average percentages of color, odor, and consistency of the feces, general animal status, duration and age average when diarrhea episodes occurred, besides globular volume (GV), and total serum protein (TSP) of newborn calves of the Negative control, Homeopathy and Zeolite groups evaluated during 60 days.

Treatments*	Color (%)					Odor (%)		Consistency (%)			General status (%)		Duration	Age	GV	TSP
	green	yellow	brown	white	grey	normal	abnormal	pasty	liquid	hard	adequate	apathetic	(day)	(day)	(%)	(g/dL)
NegControl	77.24	14.47	6.97	1.05	0.26	95.79	4.21	91.31	7.11	1.58	98.73	1.27	2.21	29.39	31.47	6.87
Homeopathy	76.59	18.08	4.42	0.78	0.13	97.04	2.96	91.82	6.36	1.82	99.62	0.38	2.48	27.44	32.76	6.62
Zeolite	78.87	15.31	4.13	1.68	0	96.44	3.56	89.77	8.85	1.37	99.70	0.3	2.57	26.43	31.67	6.49

Fonte:* No significant differences between the treatments according to the t-test ($p \leq 0.05$).

Table 2. Percentage of occurrence or not of diarrhea, mean (± standard deviation) of the number of cases of diarrhea per animal, number of antibiotic treatments per animal (Atb), weight gain (WG), cost per animal for prevention and treatment of diarrhea and cost of prevention and treatment of diarrhea by weight gain of newborn calves of the Control, Homeopathy and Zeolite groups, evaluated during 60 days.

Treatment_Diarrhea	Occurrence*	N°/animal**	Atb/animal**	WG (Kg)**	Cost/animal (US$)**	Cost/kg (US$)**
NegControl_YES	100 A	2.5 ± 1.2 A	1.2 ± 0.9 A	23.8 ± 4.2 A	4.8 ± 3.5 AB	0.2 ± 0.2 AB
Homeopathy_NO	0 B***	0 ± 0 B	0 ± 0 B	23.0 ± 7.6 A	1.4 ± 0 B	0.1 ± 0 B
Homeopathy_YES	100 A	2.4 ± 1.2 A	0.8 ± 0.4 AB	26.8 ± 5.6 A	7.7 ± 3.0 A	0.3 ± 0.1 A
Zeolite_YES	100 A	2.6 ± 1.4 A	1.0 ± 0.8 A	26.7 ± 3.1 A	5.5 ± 4.6 AB	0.2 ± 0.2 AB

* Data evaluated by the Fisher test ($p \leq 0.05$).

** Data evaluated by the SNK test ($p \leq 0.05$).

In relation to the intestinal villi of the euthanized calves, the samples of the duodenum, jejunum and ileum, observed by scanning electron microscopy, were tongue shaped, with uniform size and variation between the treatments. In the animals of the Zeolite group, there was slight preservation of the villi and crypts in the parts evaluated (Figure 1). These differences were slight, but visible when compared with the images of the Negative control group animals (Figure 2). No changes in crypts and villi were observed in animals treated with homeopathy.

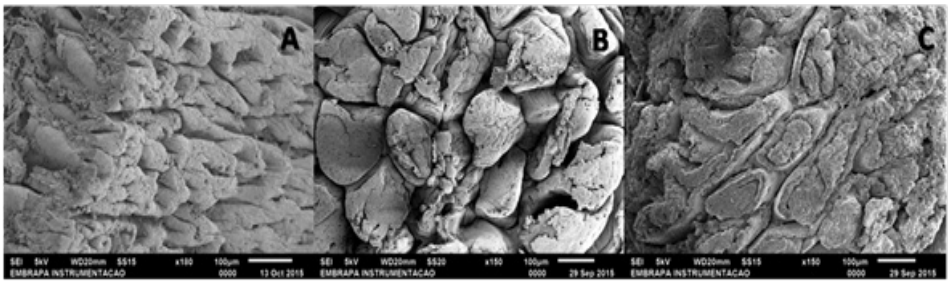

Figure 1. Scanning electron micrograph of the intestinal villi of an animal of the Zeolite group. Duodenum (x500) (A); Jejunum (x550) (B); Ileum (x500) (C).

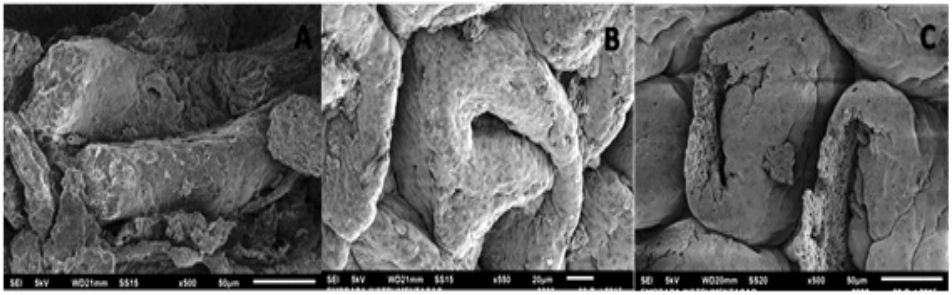

Figure 2. Scanning electron micrograph of the intestinal villi of an animal of the Negative control group. Duodenum (x180) (A); Jejunum (x150) (B); Ileum (x150) (C).

Discussion

In general, there is scarce information about the occurrence of diarrhea among dairy calves of Brazilian farms. While estimates are available, there are no official statistics about the morbidity and mortality of cattle due to different disorders (Botteon et al., 2008). However, there is an unquestionable direct impact of diarrhea episodes on the calf death rate of farms (Mohd Nor et al., 2012). Therefore, we investigated alternative methods that would be adopted to prevent diarrhea in the routine of dairy farms.

During 60 days of evaluation, the great majority of the fecal samples had normal color and pasty consistency. However, during the diarrhea episodes, these characteristics have changed as expected. The color of feces helps to diagnose the causal agent and facilitates making decisions on the necessary alterations in handling of calves (Millemann, 2009; Chagas, 2015). For example, animals suffering from colibacillosis present profuse pasty and/or liquid feces with fetid odor and yellow to white color (Coura; Lage; Heinemann, 2014). The animals presented adequate general status in almost 100% of the observations during the experimental period. It is important to note that diarrhea is not always related to apathy, especially in mild cases. An apathetic state of the animal can be related to other ailments (bovine parasitic sadness or pneumonia). The globular volume and total serum protein values were within the reference parameters established for cattle, between 24 and 46% and between 6.5 and 7.5 g/dL, respectively (Rengifo et al., 2010).

We hypothesized that the feces of the animals treated with the zeolite would be firmer, but this did not occur. The composition of the zeolite utilized was determined, and except for the presence of kaolinite, our results are the same as those reported by Martínez-Ramirez et al. (2006) for zeolite extracted from the Las Carolinas deposit, with heulandite/clinoptilolite identified as the predominant species. Papaioannou et al. (2005) noted that performance traits were affected by zeolite type, purity, particle size and the proportion of supplementation in diets. Various experiments have indicated that inclusion of natural zeolites in the diet reduces the incidence, severity, and duration of diarrhea in calves (Mumpton; Fishman, 1977; Petkova et al., 1982). Evidence exists that zeolites use can eliminate various predisposing and/or causative factors of intestinal disturbances. The administration of

zeolites to newborn animals appears to reduce the occurrence of diarrhea by increasing the passive immunity, because they increase the absorption of immunoglobulins from the colostrum by calves (Petkova et al., 1983; Vrzgula et al., 1988; Fratrić et al., 2005). In turn, Mohri; Seifi; Maleki (2008) suggested that the lesser occurrence of gastrointestinal diseases is due to the fact that zeolites absorb potentially harmful metals, or alternatively, regulate the pH in the gut. Norouzian et al. (2010), supplying 3% clinoptilolite in the initial diet of newborn lambs, observed a reduction of the incidence and severity of diarrhea. However, the clinoptilolite in the dose evaluated in our study was not efficient in preventing diarrhea.

On the other hand, the clinoptilolite was associated with positive results, because there was better preservation of the villi and crypts. Most nutrients are absorbed tn the small intestine, which is also an essential site for colonization by pathogenic agents. The proliferation of bacteria over there can cause numerous detrimental effects on the physiological processes of digestion-absorption, leading to the formation of biliary acids and biliary ketoacids, which can destroy mucous cells of the villi, leading to increased secretion by the crypt cells, causing various types of diarrhea. When the epithelial integrity is ruptured, the quantity and quality of the mucus secreted can permit the access of pathogenic agents or compounds that are harmful to the enterocytes. Therefore, the greater height of the villi and smaller depth of the crypts in the small intestine can improve the absorption of nutrients and reduce the energy expended for cell renewal. In turn, the reduction of the surface area of the villi results in less enzymatic activity, reduced digestibility and absorption of nutrients and higher sensitivity to enteric diseases or digestive disturbances (Nabuurs, 1996).

The average duration of diarrhea cases and the average age of the animals upon occurrence were statistically equal among the groups. This fact can be related to the existence of a specific etiological agent. In a study conducted in dairy herds in Canada and the United States, the researchers observed that the average age of the start of treatment for diarrhea in calves was 10 days, with an interval ranging from 0 to 71 days. Calves from birth to 2 weeks of age presented the highest incidence of diarrhea (21.2%). From 2 to 5 weeks of age, the incidence was only 1.8%, and from 5 weeks to 3 months, the occurrence was 0.35% (Windeyer et al., 2014). The average age of diarrhea in calves on a farm in the state of Rio de Janeiro was 54.2 days, and affected

animals were observed starting at 5 days of life. The highest proportion of calves with diarrhea was observed in the second week of life (39%), followed by the third week (21%) and the first week (7%) (Fagundes et al., 2014). Our data are in line with the profile found in that study, although it must be recalled that besides the etiological agent, the environmental conditions and herd management practices influence the incidence of diarrhea.

Regarding homeopathy, alternative tools are important for the sustainability of cattle breeding for organic milk production. Because of the lack of products and technologies to enable expansion of organic production in tropical countries, investigation of the efficacy of homeopathic medicines is of great interest, to enable validation and transfer of technology (Figueiredo et al., 2018). In the present study, the Homeopathic group presented 23.1% of the animals' symptom-free showing some prevention. The average cost for antibiotic treatment of cases of diarrhea was statistically the same as for the other treatments (no extra costs), i.e., Homeopathy_YES, NegControl_Yes and Zeolite_Yes. Fortuoso et al. (2018) observed that homeopathy was 50% effective in controlling bovine neonatal diarrhea and reduced the use of antibiotics. There were no differences in the animals' weight gain among the three treatments, corroborating the findings of Step et al. (2008), who did not observe weight gain differences in groups receiving 0.5% and 2% clinoptilolite, and the findings of Mohri; Seifi; Maleki (2008) in animals receiving 2% clinoptilolite in the colostrum for 48 h and 14 days. However, Ural; Ural (2017) observed more significant weight gain in Holstein calves that received 1 and 2 g of clinoptilolite/kg concerning the control group. Zarcula et al. (2010) also observed higher weight gain in animals that received 5 and 20 g of clinoptilolite/L of milk. Nevertheless, the dose evaluated in the present study (0.25% or 2.5 g/L of milk) was lower than in the mentioned studies, since, at higher concentrations, our natural mineral precipitated in the milk.

Conclusion

Although the administration of the zeolite preserved the intestinal villi, there was no effect on the prevention of diarrhea or in the weight gain. The homeopathic complex of *Arsenicum alb* 12 CH, *Nux vomica* 12CH, *Podophyllum* 12 CH, *Carbo Vegetabilis* 12CH and *China* 12 CH, presented 23.1% of efficacy in the prevention of the diarrhea episodes, no impact in its duration or in the weight gain of the calves. Although the inclusion of the homeopathic treatment does not alter the costs of the production system, the farmer needs to take into account whether the its management (daily preparation and administration) compensates the low preventive effect.

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References

BARBOSA, E. F.; FIGUEIREDO, H. C. P.; GARCIA, A. M.; LOBATO, Z. I. P.; LAGE, A. P. Rota vírus do grupo A em bezerros lactentes no estado de Minas Gerais. **Ciência Rural**, v.28, n.3, p.435-439, jul./set. 1998.

BENESI, F. J. Síndrome diarréia dos bezerros. **Revista CRMV-ES**, v.2, n.3, p.10–13, 1999.

BERCHTOLD, J. Treatment of calf diarrhea: intravenous fluid therapy. **Veterinary Clinics of North America**: Food Animal Practice, v.25, n.1, p.73–99, mar. 2009.

BOTTEON, R. de C. C. M.; BOTTEON, P. de T. L.; SANTOS JÚNIOR, J. de C. B.; PINNA, M. H.; LÓSS, Z. G. Frequency of diarrhea in dairy calves submitted to different breeding conditions in the border of Rio de Janeiro and Minas Gerais states, Brazil. **Brazilian Journal of Veterinary Research and Animal Science**, v.45, n.2, p.153–160, 2008.

CHAGAS, A. C. de S. (Ed.). **Diarréia em bezerros leiteiros lactantes**: a doença e o manejo em diferentes unidades da Embrapa. São Carlos, SP: Embrapa Pecuária Sudeste, 2015. (Embrapa Pecuária Sudeste. Documentos, 120).

CHO, Y.-il; YOON, K.-Jin. An overview of calf diarrhea: infectious etiology, diagnosis, and intervention. **Journal of Veterinary Science**, v.15, n.1, p.1–17, mar. 2014.

COURA, F. M.; LAGE, A. P.; HEINEMANN, M. B. Patotipos de *Escherichia coli* causadores de diarreia em bezerros: uma atualização. **Pesquisa Veterinária Brasileira**, v.34, n.9, p.811–818, set. 2014.

ECONOMOU, V.; GOUSIA, P. Agriculture and food animals as a source of antimicrobial-resistant bacteria. **Infection and Drug Resistance**, v.8, p.49-61, apr. 2015.

FAGUNDES, T. F.; VIDAL, L. G. P.; ALVES, P. A. M.; TASSINARI, W. de S.; COELHO, S. de M. de; MENEZES, R. de C. A de A.; FONSECA, A. H. da; PEREIRA, M. J. S. Análise descritiva da diarreia em uma coorte de bezerras criadas em sistema de casinhas até cem dias de idade, Município de Piraí, Estado do Rio de Janeiro, Brasil. **Semina Ciências Agrárias**, v.35, n.4, p.1895–1912, 2014.

FIGUEIREDO, A.; FANTATTO, R. R.; AGNOLON, I. C.; LOPES, L. G.; OLIVEIRA, P. R. de; MATHIAS, M. I. C.; ALVES, T. C.; BARIONI JUNIOR, W.; CHAGAS, A. C. de S. In vivo study of a homeopathic medicine against Rhipicephalus (Boophilus) microplus in dairy cow. **Revista Brasileira de Farmacognosia**, v.28, p.207-213, mar./abr. 2018.

FORTUOSO, B. F.; VOLPATO, A.; RAMPAZZO, L.; GLOMBOWSKY, P.; GRISS, L. G.; GALLI, G. M.; STEFANI, L. M.; BALDISSERA, M. D.; FERREIRA, E. B.; MACHADO, G.; SILVA, A. S. da. Homeopathic treatment as an alternative prophylactic to minimize bacterial infection and prevent neonatal diarrhea in calves. **Microbial Pathogenes**, v.114, p.95–98, jan. 2018.

FRATRIĆ, N.; STOJIĆ, V.; JANKOVIĆ, D.; SAMANC. H.; GVOZDIĆ. D. The effect of a clinoptilolite based mineral adsorber on concentrations of immunoglobulin g in the serum of newborn calves fed different amounts of colostrum. **Acta Veterinaria (Beograd)**, v.55, n.1, p.11–21, 2005.

FRERKING, H.; ROMANSKY-RIEKEN, M. E. Erfahrungen mit dysenteral bei durchfallerkrankungen junger kälber [Experiences with dysenteral in diarrhea of young calves]. **Der Praktische Tierarzt**, v.63, p.17-18, 1982.

HAMMON, H. M.; SCHIESSLER, G.; NUSSBAUM, A.; BLUM, J. W. Feed intake patterns, growth performance, and metabolic and endocrine traits in calves fed unlimited amounts of colostrum and milk by automate, starting in the neonatal period. **Journal of Dairy Science**, v.85, n.12, p. 3352–3362, dec. 2002.

HUR, T. Y.; JUNG, Y. H.; CHOE, C. Y.; CHO, Y. I.; KANG, S. J.; LEE, H. J.; KI, K. S.; BAEK, K. S.; SUH, G. H. The dairy calf mortality: the causes of calf death during ten years at a large dairy farm in Korea. **Korean Journal of Veterinary Research**, v.53, n.2, p.103-108, 2013.

JASPER, J.; WEARY, D. M. Effects of ad libitum milk intake on dairy calves. **Journal of Dairy Science**, v.85, n.11, p.3054–3058, nov. 2002.

KAYNE, S.; RAFFERTY, A. The use of Arsenicum album 30c to complement conventional treatment of neonatal diarrhea ('scours') in calves. **Br. Homoeopath J.,** v.83, n.4, p.202-204, 1994.

LANGONI, H.; LINHARES, A. C.; AVILA, F. A.; SILVA, A. V. da; ELIAS, A. O. Contribution to the study of diarrhea etiology in neonate dairy calves in São Paulo state, Brazil. **Bras. J. Vet. Res. Anim. Sci.**, v.41, n.5, p.313-319, sep./oct. 2004.

LUNA, F. J.; SCHUCHARDT, U. Modificação de zeólitas para uso em catálise. **Química Nova**, v.24, n.6, p.885–892, nov./dec. 2001.

MARTÍNEZ-RAMÍREZ, S.; BLANCO-VARELA, M. T.; EREÑA, I.; GENER, M. Pozzolanic reactivity of zeolitic rocks from two different Cuban deposits: characterization of reaction products. **Applied Clay Science**, v.32, n.1-2, p.40-52, apr. 2006.

MILLEMANN, Y. Diagnosis of neonatal calf diarrhea. **Revue Medicine Veterinaire**, v.60, n.8-9, p.404-409, 2009.

MOALLEM, U.; WERNER, D.; LEHRER, H.; ZACHUT, M.; LIVSHITZ, L.; YAKOBY, S.; SHAMAY, A. Long-term effects of ad libitum whole milk prior to weaning and prepubertal protein supplementation on skeletal growth rate and first-lactation milk production. **Journal of Dairy Science**, v.93, n.6, p.2639-2650, june 2010.

MOHD NOR, N.; STEENEVELD, W.; MOURITS, M. C. M.; HOGEVEEN, H. Estimating the costs of rearing young dairy cattle in the Netherlands using a simulation model that accounts for uncertainty related to diseases. **Preventive Veterinary Medicine**, v.106, n.3-4, p.214-224, oct. 2012.

MOHRI, M.; SEIFI, H. A.; MALEKI, M. Effects of short-term supplementation of clinoptilolite in colostrum and milk on the concentration of some serum minerals in neonatal dairy calves. **Biological Trace Element Research**, v.123, n.1-3, p.116-123, feb. 2008.

MOTA, R. A.; SILVA K. P. C.; RIBEIRO T. C. F.; RAMOS G. A. B.; LIMA E. T.; SILVA L. B. G.; ZÜNIGA C. E. A. Eficácia do Nuflor no tratamento de diarreias em bezerros e leitões. **A Hora Veterinária**, v. 118, p.21-24, 2000.

MUMPTON, F.A.; FISHMAN, P. H. The application of natural zeolites in anima science and aquaculture. **Journal of Animal Science**, v.45, n.5, p.1188-1203, nov. 1977.

NABUURS, M. J. A. Microbiological, structural and function changes of the small intestine of pigs at weaning. **Pig News and Information**, v.16, n.3, p.93-97, 1996.

NOROUZIAN, M. A.; VALIZADEH, R.; KHADEM, A. A.; AFZALZADEH, A.; NABIPOUR, A. The effects of feeding clinoptiloliteon hematology, performance, and health of newborn lambs. **Biological Trace Element Research**, v.137, p.168-176, 2010.

PALIK, E. S. Specific surface area measurements on ceramic powders. **Powder Technology**, v.18, n.1, p.45–48, sep./oct. 1977.

PAPAIOANNOU, D.; KATSOULOS, P. D.; PANOUSIS, N.; KARATZIAS, H. The role of natural and synthetic zeolites as feed additives on the prevention and/or the treatment of certain farm animal diseases: a review. **Microporous Mesoporous Materials**, v.84, n.1-3, p.161-170, sep. 2005.

PETKOVA, E.; VENKOV, T.; CHUSHKOV, P.; DZHUROV, A.; STEFANOV, T. S. Bulgarian potassium-calcium zeolite in the prophylaxis of digestive tract disorders in calves. **Vet. Med. Nauki**, v.19, n.4, p.55-62, 1982.

PETKOVA, E.; VENKOV, T.; STANCHEV, K. Effects of Bulgarian potassium-calcium zeolites on the assimilation of macro and trace elements in lambs. **Vet. Med. Nauki**, v.20, n.8, p.36-40, 1983.

PRASAI, T. P.; WALSH, K. B.; BHATTARAI, S. P.; MIDMORE, D. J.; VAN, T. T. H.; MOORE, R. J.; STANLEY, D. Zeolite food supplementation reduces abundance of enterobacteria. **Microbiological Research**, v.195, p.24-30, jan. 2017.

RAETH-KNIGHT, M.; CHESTER-JONES, H.; HAYES, S.; LINN, J.; LARSON, R.; ZIEGLER, D.; ZIEGLER, B.; BROADWATER, N. Impact of conventional or intensive milk replacer programs on Holstein heifer performance through six months of age and during first lactation. **Journal of Dairy Science**, v.92, n.2, p.799-809, feb. 2009.

REGITANO, J. B.; LEAL, R. M. P. Comportamento e impacto ambiental de antibióticos usados na produção animal brasileira. **Revista Brasileira de Ciência do Solo**, v. 34, n.3, p.601-616, 2010.

RENGIFO, S. A.; SILVA, R. A.; BOTTEON, R. C. C. M.; BOTTEON, P. T. L. Hemograma e bioquímica sérica auxiliar em bezerros mestiços neonatos e ocorrência de enfermidades. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v.62, n.4, p.993-997, ago. 2010.

SAS Institute (Cary, NC). **SAS version 9.4, for windows**. Cary: SAS Institute, 2012.

STEP, D. L.; LITHERLAND, N. B.; BURCIAGA-ROBLES, L. O.; BRESHEARS, M. A.; KREHBIEL, C. R.; CONFER, A. W.; FULTON, R. W.; MORGAN, G. L.; THORNSBERRY, R. M.; FASSIG, S. M. Clinical observations, biochemical data and postmortem and histopathologic findings in young dairy calves fed zeolite clinoptilolite binder combined with milk replacer. **American Journal of Veterinary Research**, v.69, n.12, p.1587-1594, dec. 2008.

TEIXEIRA, M. Z. Homeopatia: ciência, filosofia e arte de curar. **Revista de Medicina**, v.85, n.2, p.30-43, 2006.

URAL, D. A.; URAL, K. Efectos de la suplementación clinoptilolita corto plazo sobre la ganancia de peso en becerros Holstein. **Journal MVZ Córdoba**, v.22, n.1, p.5631-5637, ene./apr. 2017.

VERDIER, K. de; OHAGEN, P.; ALENIUS, S. No effect of a homeopathic preparation on neonatal calf diarrhea in a randomised double-blind, placebo-controlled clinical trial. **Acta Veterinaria Scandinavica**, v.44,n.1-2, p.97-101, 97, 2003.

VIEIRA, E. C.; KAMOGAWA, M. Y.; LEMOS, S. G.; NÓBREGA, J. de A.; NOGUEIRA, A. R. de A. Decomposição de amostras de solos assistida por radiação microondas: estratégia para evitar a fomação de fluoretos insolúveis. **Revista Brasileira de Ciência do Solo**, v.29, n.4, p.547-553, jul./ago. 2005.

VRZGULA, L.; PROSBOVA, M.; BLAZOVSKY, J.; JACOBI, U.; SCHUBERT, T.; KOVAC, G. The effect of feeding natural zeolite on indices of the internal environment of calves in the postnatal period. In: KALLO, D.; SHERRY, H. S. (Eds.). **Occurrence, properties and utilization of natural zeolites**. Budapest: Akademiai Kiado, 1988. p.747-752.

WINDEYER, M. C.; LESLIE, K. E.; GODDEN, S. M.; HODGINS, D. C.; LISSEMORE, K. D.; LEBLANC, S. J. Factors associated with morbidity, mortality, and growth of dairy heifer calves up to 3 months of age. **Preventive Veterinary Medicine**, v.113, n.2, p.231-240, feb. 2014.

ZARCULA, S.; TULCAN, C.; SAMANC, H.; KIROVSKI, D.; CERNESCU, H.; MIRCU, C. Clinical observations in calves fed colostrum supplemented with clinoptilolite. **Lucrari Stintifice Medicina Veterinara**, v.43, n.2, p.64-69, 2010.

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